Middle East Journal of Applied Science & Technology
Vol.5, Iss.3, Pages 22-41, July-September 2022

Vector Bionomics, Potential Vectors, Susceptibility and Bio-efficacy of LLINs Nets on *Anopheles* Mosquitoes in Nanyin Village Bunmouk Township Sagaing Region in Myanmar

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DOI: https://doi.org/10.46431/MEJAST.2022.5303

Article Received: 10 May 2022 *Article Accepted: 19 July 2022* *Article Published: 30 August 2022*

ABSTRACT

Nanyin village is a malaria endemic area consisting of Bunmouk Township Sagaing Region in Myanmar. Ohnpinkone and Bwedarkone wards were chosen for measuring vector bionomics, potential vectors, susceptibility and bio-efficacy of long lasting insecticidal nets (LLINs) (due to high prevalence of *P. falciparum*) in both wards from December 2019 to October 2020. A total of 3236 and 3019 *Anopheles* mosquitoes consist of 12 species were collected from both Ohnpinkone and Bwedarkone wards. *Anopheles barbirostris* was found highest number (1088 and 820) followed by *An. philippinensis* (267 and 605) in both areas. Higher number of *Anopheles* mosquitoes were collected by Cattle bait catching method followed by Indoor Light traps method. The highest was collected in October in Ohnpinkone and September in Bwedarkone. In Ohnpinkone, the highest was collected in April by outdoor and August in indoor light trap and March by outdoor and June by indoor light traps in Bwedarkone. Higher number of main vectors of *An. minimus* and *An. dirus* were collected in Ohnpinkone than Bwedarkone and highest number of *An. minimus* was collected by cattle bait (77) followed by indoor light method (72) in Ohnpinkone. Only 11and 3 *An. dirus* were collected, of this 9 and 1 number were collected by indoor light traps method from both wards. One *An. minimus* from Ohnpinkone was found sporozoite positive (0.47%). Main vector *An. minimus* was collected highest in April in Ohnpinkone and June in Bwedarkone. Susceptibility status of primary and secondary vectors were found susceptible to Pyrethroid insecticides. Bio-efficacy of all tested LLINs nets were found lower efficacy 20% and 63.33% in both wards. Main vector of *An. minimus* was collected year round in large number in both areas as well as sporozoite positive and *An. dirus* was available in both areas. Therefore, both areas are highly malaria risk areas and need to proper treatment and control measure using RIS and supply new LLINs nets or retreatment with deltamethrin to old nets according to WHO guideline to ovoid malaria transmission.

Keywords: Bionomics, Susceptibility, Sporozoite, LLINs, *Anopheles*, Main vector, *An. minimus*, Bio-efficacy, Pyrethroid, Deltamethrin insecticide.

1. Introduction

Malaria is one of the major communicable disease-causing high mortality and morbidity among population in malaria endemic areas where tropical and sub-tropical climate zone such as Asia, Africa and South Saharan country. Malaria is high in south East Asian Regions including Myanmar. Myanmar government has committed to eliminate malaria by 2030. The national framework for malaria elimination released by the Government of Myanmar plans to achieve this goal through strategic planning in a phased manner [1]. India also plan to eliminate malaria in 2030 releases by government of India [2].

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Since vector control is a major component of disease management and vector elimination, it requires a detailed understanding of the biology and bionomics of malaria vectors exhibiting specific distribution pattern [2]. As per the World Malaria Report 2018, in 2017 80% of the global malaria burden was borne by 15 countries in sub-Saharan Africa and India. India (with 4% malaria cases) is one of the five countries that contributed 50% malaria cases worldwide [3]. In line with global developments in achieving the elimination of malaria in different countries, Myanmar adopted the goal of eliminating malaria by 2030 and aims to eliminate *P. falciparum* malaria by 2025 in line with the Greater Mekong Sub-Region Malaria Elimination Strategy. Significant progress has been made over recent years in reducing malaria morbidity and mortality in Myanmar. Malaria morbidity declined by 72% and mortality dropped by 95% in 2016 in comparison with 2012, reflecting significant improvement in access to diagnosis, treatment and prevention of malaria. Five States or Regions of Myanmar are already in elimination phase of malaria. All States and Regions are expected to follow suit in the years ahead [4]. Previously malaria is a main public health problem in Myanmar. Although now morbidity and mortality of malaria is reducing from 4% to 0.0001% by 100000 populations 2011 to 2018 [5]. Malaria is disappeared in most part of Country but malaria is remaining endemic in hard to reach areas and border areas [6]. Four malaria parasites infected to human in Myanmar, *P. falciparum* is a deadly parasite. Previously, 85% of the malaria cases were found *P. falciparum* and *P. vivax* was occurred 15%. *Plasmodium vivax* is gradually rising in Myanmar [7]. Now out of which about half of the cases were *Plasmodium vivax* (*Pv*). The disease control efforts globally including Myanmar have depended largely on the use of pyrethroid-based insecticide-treated nets and indoor residual spraying strategy (IRS). These interventions have greatly contributed to significantly reduce the burden of malaria in some parts of Myanmar and significantly reduce the burden of malaria in the GMS where the disease elimination is a priority [1,8]. The efficacy of any vector control interventions is strongly influenced by the ecology and behaviour of malaria vectors [9]. The vector bionomics and susceptibility of malaria vectors in malaria endemic areas are very important to destroy the vectors of malaria.

The disease of the malaria is transmitted by biting of vector female Anopheline mosquito species. Out of 37 *Anopheles* species found in Myanmar, *An. dirus* and *An. minimus* are major vectors of malaria and *An. annularis* is a local vector of malaria in Rakhine State and *An. sundiacus* is a vector of malaria in coastal areas in Myanmar [10]. Although *An. annularis, An. maculatus, An. aconitus* and *An. philippinensis* are secondary vectors of malaria in Myanmar. In India the six species viz., *Anopheles culicifacies, An. stephensi, An. minimus, An. sundiacus, An. fluviatilis* and *An. dirus* are major vectors of malaria and three species viz., *An. annularis, An. philippinensis* (nivipus) and *An. varuna* are minor vectors of malaria and *Anopheles culicifacies* sibling as A, C, D and E are the major vectors and B is a non-vector of malaria in India [11,12]. Non vector of *Anopheles culicifacies* sibling species B was abundantly found in Poukkaung and Goepinkauk Townships Bago Region and Sedawgyi Township in Mandalay Region as well as Myothit Township Magwe Region [13-15]. In Pakistan and Sri Lanker *Anopheles culicifacies* sibling species B carried malaria parasites [16,17].

Anopheles dirus D and *An. minimus* A are abundantly found in Thabwewa village Bago Region, Mudon Township Mon State, Yephyu Township, Taninthayi Region and Katinehtit village Kamamaung Township, Kayin State, and high density of sibling species *An. minimus* A was collected in Phyu Township Bago Region and Pyinoolwin

Township Mandalay [15,18-22]. In the context of elimination, entomological aspects of malaria transmission are crucial in order to devise, and implement effective, scalable and locally-adapted vector control interventions [23].

Nanyin village is a remote and hard to reach area, it is 40-50 miles away from Bunmouk City. There are total of four wards, Ohnpinkone and Bwedarkone wards are included in Nanyin village. Total of population is about 5000 populations in village. Most of the population are farmers remaining are health staff, school teacher and some are working in Gold mines. One monastery and one middle school is situated. The area of the Nanyin is a hilly area and there is no pucker roads. One creek is across in the village. So many creeks crossed between Bunmouk and Nanyin village. According to Hospital report, malaria cases were frequently coming from this area. Therefore, there is a need to control malaria morbidity as well as main vectors of malaria. The study determined monthly malaria vectors occurrence in Ohnpinkone and Bwedarkone wards. Because in preliminary study found 10 *Plasmodium falciparum* malaria cases in Ohnpinkone and 1 *P. falciparum* cases in Bwedarkone ward. The study aims to determine the vector biology, potential vector and susceptibility status of collected *Anopheles* mosquitoes from both study areas in Nanyin village Bunmouk Township Sagaing Region.

2. Materials and Methods

Study areas: The study was conducted in Ohnpinkone and Bwedarkone wards in Nanyin village Bunmouk Township Sagaing Region in Myanmar. Where the morbidity of malaria is high.

2.1. About the study areas

Nanyin village is a remote and hard to reach area, it is 40-50 miles away from Bunmouk City. There are total of four wards, Ohnpinkone and Bwedarkone wards are included in Nanyin village. Total population is about 5000 populations in village. Most of the population are farmers remaining are health staff, school teacher and some are working in Gold mines. One monastery and one middle school are situated. The area of the Nanyin is a hilly area and there are no pucker roads. One creek is across in the village. So many creeks cross between Bunmouk and Nanyin village. Malaria morbidity is found every month in this area. Therefore, there is a need to control malaria morbidity as well as man vector contact. Study determined the vectors bionomics, monthly occurrence of potential vectors, susceptibility and bio-efficacy status of *Anopheles* mosquitoes in Ohnpinkone and Bwedarkone wards in Nanyin villages. According to the Bunmouk Township hospital records, malaria cases were frequently come from Nanyin village to admit hospital.

Study area and period: Entomological survey was conducted to better understand the distribution, abundance behaviour and susceptibility of major malaria vectors (*Anopheles spp.*) in Nan Yin village Bunmouk Township Sagaing Region from December 2019 to November 2020 for one year.

2.2. Study design

Descriptive study design was used.

2.3. Mosquitoes collection

Anopheles mosquitoes were collected using cattle bait big mosquito trap nets called Kanda net (K-net) and Indoor and outdoor CDC light traps collection method was used for 18:00 to 6:00 hours of next day. Hourly catch was

done from 18:00 to 24:00 hours using WHO sucking tube. Indoor and outdoor CDC light trap collection was done in ten selected households from 18:00 to 6:00 hours for 5days.

2.4. Larval surveys

Larval surveys for identification of breeding sites were conducted in and around three kilo-meters away from the study villages. For larval detection water pools, domestic wells, stream, creeks, sand pools and all different types of water holding places as water pockets, coconut shells, discarded tins and utensils bamboo stumps including foot print of animals were examined by 3 Dips/water holding place [24]. The captured larvae and pupae were put in labeled plastic bags and brought back to the laboratory for species identification and colonization.

2.5. Mosquito species identification

Collected *Anopheles* mosquitoes and adult emerged from larval survey were identified according to different identification keys [25,26,27].

2.6. Vector incrimination

Head and thorax of vector mosquitoes were dissected for *Plasmodium* sporozoites. Enzyme Linked Immunosorbent assay (ELISA) test for circumsporozoite antigen detection was supplemented according to Wirtz et al., [28] for vector incrimination study.

2.7. Insecticide susceptibility test

Insecticide susceptibility tests (WHO test kit): Collected adult female *Anopheles* mosquitoes from the entomological survey were tested for measurement of insecticide susceptibility level using WHO test kits and standard procedures [29]. The efficacy of insecticides which are commonly used for vector control in malaria endemic areas namely as Permethrin 0.75%, Cyfluthrin 0.15% and Deltamethrin 0.05% impregnated paper with WHO test kits were provided.

2.7.1. Procedure

5 to10 fields collected *Anopheles* mosquitoes were introduced in WHO insecticide impregnated paper attached plastic tube (WHO test kit) by sucking tube and exposed for 1 hour. After one hour of exposing the mosquitoes were then removed from the plastic tubes and placed in clean plastic tubes without paper with 10% glucose soaked cotton and moisture were maintained by water soak dump towel. Percentage of knockdown was measured after 60 minutes' exposure and effective mortality was assessed after 24 hours' exposure. Two replicate testing were done to confirm the susceptibility of mosquitoes. If the quantity of collected mosquitoes was not more than 10 while we used pool mosquito samples to test insecticides susceptibility. Susceptibility of mosquitoes were determined according to WHO [29].

2.8. Bio-efficacy test (Cone test method)

Determination of insecticide persistence – Bioassays were carried out using the World Health Organization cone test method [30] on distributed long lasting insecticidal Nets(LLINs) in both Ohnpinkone and Bwedarkone wards to monitor the persistent effect of the insecticide treated LLINs net. Ten LLINs nets each of different brands were

randomly collected in both wards and Cone bioassays test was conducted to determine the persistent bio-efficacy of LLINs nets. For comparison, tests were conducted in parallel on one untreated net obtained from the villagers. Ten sets of 10 wild-caught fully fed female *Anopheles* mosquitoes were exposed to the LLINs nets for 3 min and the mortality was recorded after 24 hours. Primary and Secondary vectors of malaria were used for the bioassay tests.

2.9. Data analysis

Data entry and collected monthly malaria and mosquito data were analyzed by using Microsoft Excel software. Mosquito density, main vector of indoor and outdoor light traps, mosquito susceptibility and mortality were calculated in percent.

3. Results

Table 1. *Anopheles* mosquitoes collected by different methods in two Control and test wards in Nanyin village

Species	Ohnpinkone Ward (Test ward)						Bwedarkone (Control ward)					
	CB	Outdoor LT	In door LT	Total	Density	Sporozoite positivity%	CB	Outdoor LT	Indoor LT	Total	Density	Sporozoite positivity
An. Kochi	175	5	14	194	6	0	130	3	6	139	4.61	0
An. barbirostris	962	42	84	1088	33.63	0	799	10	11	820	27.17	0
An. hyrcanus	68	7	7	82	2.53	0	48	0	1	49	1.62	0
An. splendidus	217	31	10	258	7.98	0	109	28	10	147	4.87	0
An. minimus	77	65	72	214	6.62	1 (0.47)	60	36	67	163	5.40	0
An. varuna	160	11	40	211	6.52	0	86	11	18	115	3.81	0
An. mculatus	109	11	16	136	4.20	0	42	4	4	50	1.66	0
An. Jamesi	65	32	2	99	3.06	0	36	2	2	40	1.33	0
An. aconitus	108	16	24	148	4.57	0	147	10	15	172	5.70	0
An. stephensi	3	1	18	22	0.68	0	0	0	0	0	0.00	0
An. candidiensis	28	7	3	38	1.17	0	18	4	4	26	0.86	0
An. pallidus	47	2	10	59	1.82	0	150	1	0	151	5.00	0
An. theobaldi	0	0	0	0	0.00	0	1	0	0	1	0.03	0
An. Annularis	72	8	9	89	2.75	0	99	7	7	113	3.74	0
An. philippinensis	193	33	41	267	8.25	0	568	20	17	605	20.05	0

An. gigus	4	0	0	4	0.12	0	3	0	0	3	0.10	0
An. vagus	134	24	90	248	7.67	0	300	17	20	337	11.17	0
An. dirus	0	2	9	11	0.34	0	2	0	1	3	0.1	0
An. culicifacies	10	3	7	20	0.62	0	13	0	2	15	0.50	0
An. willmori	2	0	21	23	0.71	0	0	0	40	40	1.33	0
An. tesselatus	7	0	4	11	0.34	0	6	1	0	7	0.23	0
An. subpictus	9	1	4	14	0.43	0	22	0	1	23	0.76	0
Total	2450	301	484	3236	100	1 (0.03)	2639	154	225	3019	100	0

Table 1 Shows that in comparison the highest number of vector *An. minimus* was found n= 77 in cattle bait collection followed by n=72 in indoor light traps collection lowest was observed n=65 in outdoor collection in Ohnpinkone ward, In Bwedarkone ward the highest number of main vector *An. minimus* was found n=67 in outdoor light traps collection followed by n=60 in cattle bait collection lowest was observed n=36 in indoor collection. One *An. minimus* was found circumsporozoite positive in Ohnpinkone wards (0.47%) and not circumsporozoite positive was observed in Bwedarkone ward. Although *An. barbirostris* was abundantly found in both Ohnpinkone and Bwedarkone wards followed by *An. philippinensis* n=605 in Bwedarkone and Ohnpinkone wards. Other secondary vectors such as *An. aconitus, An. annularis, An. culicifacies, An. maculatus and An. kochi* were found high number in both areas. Highest density of *An. barbirostris* 33.63% was collected followed by *An. philippinensis* 8.25% and lowest was observed *An. gigus* 0.12% in Ohnpinkone wards and in Bwedarkone the highest density of *An. barbirostris* 27.17% was collected followed by *An. philippinensis* 20.05% and lowest was observed *An. gigus* 0.1%.

Table 2. Monthly collection of *Anopheles* mosquitoes in Ohnpinkone and Bwedarkone wards in Nanyin village

Places	Monthly collection of *Anopheles* mosquitoes										
	December	**Jan**	**Feb**	**Mar**	**Apr**	**Jun**	**July**	**Aug**	**Sept**	**Oct**	**Total**
Ohnpinkone Ward	456	65	88	305	175	397	412	455	316	567	3236
Bwedarkone Ward	74	51	134	139	83	352	656	297	727	502	3019

Table 2 shows that monthly occurrence of Anopheline mosquitoes in Ohnpinkone and Bwedarkone wards. In Bwedarkone ward, the highest number of *Anopheles* mosquitoes were collected in September (n=727) followed by July (n=656) and lowest was observed in January (n=51). In Ohnpinkone ward, the highest number of mosquitoes were collected in October (n=567) followed by August (n=455) and lowest was collected in January (n=65).

Middle East Journal of Applied Science & Technology
Vol.5, Iss.3, Pages 22-41, July-September 2022

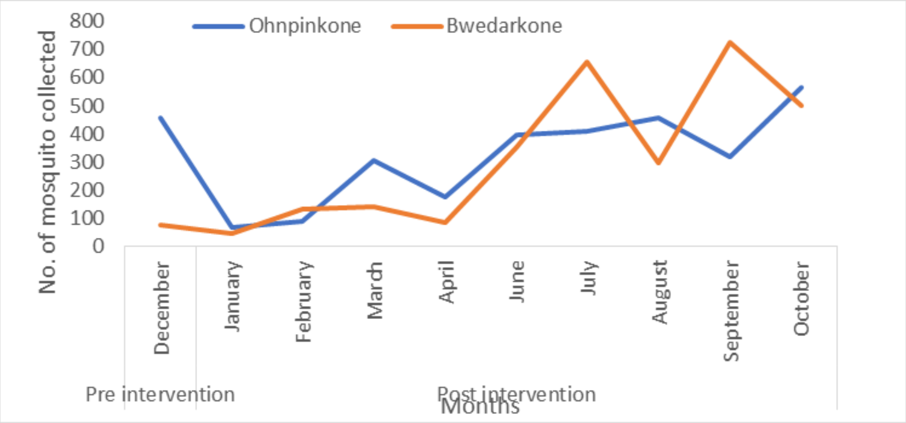

Fig.1. Monthly collected *Anopheles* mosquitoes in Ohnpinkone and Bwedarkone wards in Nanyin Township

Fig.1 shows that after impregnation of mosquito nets, *Anopheles* mosquito density was fall down from January to April and then the density was gradually rising to October in both wards.

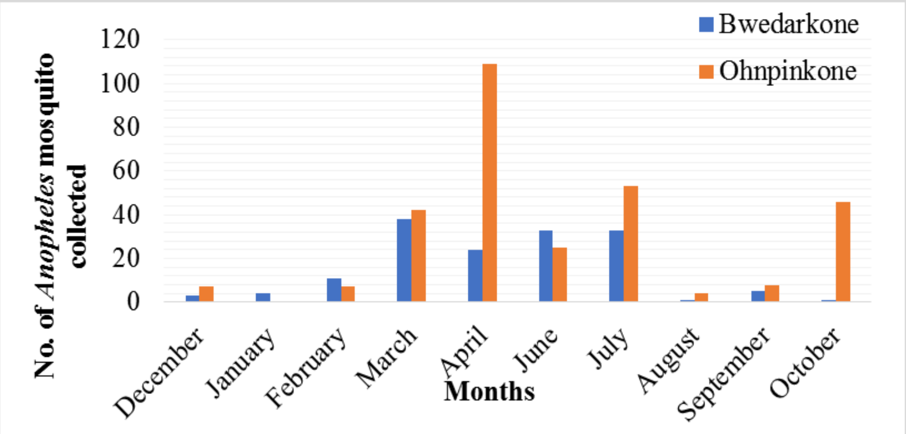

Fig.2. Comparison of Outdoor Light Traps caught in Bwedarkone and Ohnpinkone wards

Fig.2 shows that comparison of monthly *Anopheles* mosquitoes distributed with each ward, the highest number of *Anopheles* mosquitoes were collected in Ohnpinkone than Bwedarkone wards in April. In January, *Anopheles* mosquitoes have not caught by light traps catching method in Ohnpinkone ward. In Bwedarkone the highest number of Anopheles mosquitoes were collected in March followed by June and July and lowest was observed in August and October.

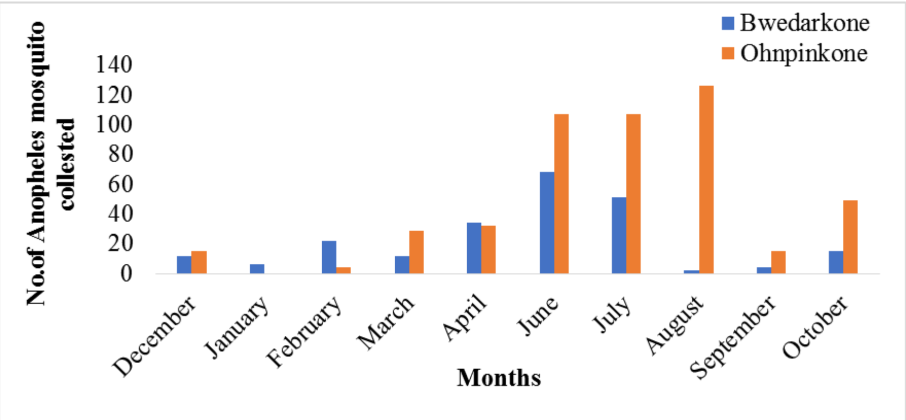

Fig.3. Comparison of indoor Light Traps caught in Bwedarkone and Ohnpinkone wards

Fig.3 shows that indoor catches *Anopheles* mosquitoes were found gradually high from January to August in both wards although the highest number of *Anopheles* was collected by indoor light traps in August and followed by June and July and lowest number was observed in February. In d Bwedarkone ward the highest *Anopheles* mosquitoes were collected in June followed by July and lowest was found in August. When compared with indoor mosquitoes in both wards Ohnpinkone was found higher than Bwedarkone except January and February indoor catch.

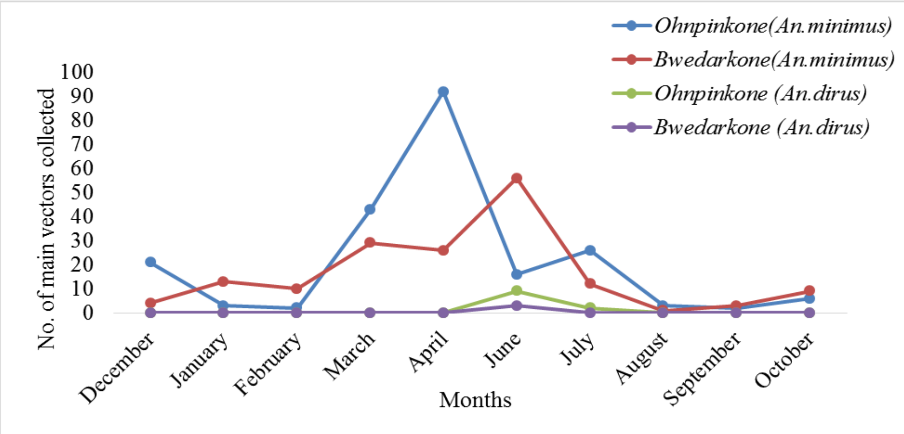

Fig.4. Monthly occurrence of main vectors *An. minimus* and *An. dirus* collected by different methods in Ohnpinkone and Bwedarkone wards

Fig.4 shows that monthly collection of main vectors *An. minimus* and *An. dirus* by different methods in Ohnpinkone and Bwedarkone wards were found that *An. minimus* was collected in every month in both areas. In Ohnpinkone ward *Anopheles minimus* was found suddenly increased from February and highest pick was found in April then the density of *An. minimus* was decreased to June, then secondary small peak was observed in July after that gradually decreased to October. In Bwedarkone ward *An. minimus* was collected in all months, although gradually increased from December to June then suddenly decreased to August and continuously decreased to October. *Anopheles dirus* was disappeared from December to April and appeared on June to July and high pick was found in June in both areas, although very low number of *An. dirus* was collected, 9 *An. dirus* from Ohnpinkone and 3 *An. dirus* from Bwedarkone were collected.

Table 3. Larval survey in different breeding sources in Ohnpinkone and Bwedarkone wards

Breeding sources	No. of search sites	No. of +ve sites	Species of larvae	Density	No. of search	No. of +ve sites	Species of larvae	Density
Wells	12	9	46 *An. minimus*	80 (16.60%)	12	4	11 *An. minimus*	18 (6.84%)
			23 *An. dirus*				5 *An. dirus*	
			11 *An. maculatus*				2 *An. maculatus*	
Creek(Back)	35	12	10 *An. splendidus*	55 (11.41%)	32	11	9 *An. splendidus*	21 (7.99%)
			18 *An. kochi*				5 *An. kochi*	

			16 *An. maculatus*				2 *An. varuna*	
			11 *An. varuna*				5 *An. jamesi*	
Valley	12	7	9 *An. minimus*	61 (12.66%)	4	2	4*An. minimus*	21 (7.99%)
			13 *An. varuna*				3 *An. varuna*	
			22 *An. barbirostris*				3 *An. splendidus*	
			3 *An. aconitus*				3 *An. aconitus*	
			8 *An. maculatus*				8 *An. maculatus*	
			3 *An. jamesii*					
			3 *An. splendidus*					
Rice field	54	38	13 *An. minimus*	136 (28.22%)	52	36	2 *An. minimus*	109 (41.45%)
			21 *An. maculatus*				17 *An.maculatus*	
			42 *An. barbirostris*				33 *An. barbirostris*	
			17 *An. aconitus*				1 *An. splendidus*	
			23 *An. philippinensis*				18 *An. philippinensis*	
			20 *An. varuna*				27 *An. aconitus*	
							11 *An. varuna*	
Sand pools	6	5	16 *An. culicifacies*	64 (13.28%)	13	6	5 *An. culicifacies*	39 (14.83%)
			25 An. minimus				23 *An. minimus*	
			16 *An. maculatus*				*11 An. maculatus*	
			7 *An. philippinensis*					
Water pools	8	6	12 *An. minimus*	86 (17.84%)	6	4	*8 An. minimus*	55 (20.91%)
			10 *An. maculatus*				7*An.maculatus*	
			34 *An. barbirostris*				21 *An. barbirostris*	
			11 *An. aconitus*				9 *An. aconitus*	
			12 *An. philippinensis*				7 *An. philippinensis*	
			7 *An. varuna*				3 *An. varuna*	
	127	77 (60.63%)		482 (100%)	119	63 (52.94%)		263 (100%)

Table 3 shows that larval survey for breeding sources were detected in different water holding sources such as Water wells, Creeks, Valleys, Rice fields Sand pools and water pools in both areas. A total of 127 and 119 water sources were detected for Anopheles larvae in both Ohnpinkone and Bwedarkone wards of this 77(60.63%) and 63(52.94%) of the water sources were *Anopheles* larvae positive. Highest density of *Anopheles* larvae was found in Rice field water sources followed by136(28.22%) and 109(41.45%) followed by water pools 86(17.84%) and 55(20.19%) and lowest was observed in creeks 55(11.41%) and wells 18(6.84%) in both Ohnpinkone and Bwedarkone wards respectively. Main vector *An. dirus* larvae were collected in only water wells, higher number 23 larvae from Ohnpinkone than 5larvae from Bwedarkone wards (5). Although main vector *An. minimus* larvae were collected in all water sources except in creeks in both areas. Highest number of *An. minimus* larvae were collected in wells followed by sand pools lowest was collected in valleys in Ohnpinkone ward and in Bwedarkone ward, the highest number of *An. minimus* larvae were collected in sand pools (23) followed by water wells (11) and lowest was observed in rice fields (2). Secondary vector *An. maculatus* were collected in all water sources from Ohnpinkone and all water sources expect in creeks in Bwedarkone. Other secondary vectors as *An. aconitus, An. culicifacies*, *An. philippinensis* larvae were collected in different water sources in both areas.

Table 4. Susceptibility status of collected main and secondary vectors of *Anopheles* mosquitoes tested with WHO test kits in Ohnpinkone area

Species	Number of mosquitoes	WHO [29] recommended insecticides impregnated paper and test kit	susceptible
An. minimus	30	Deltamethrin 0.05% Permethrin 0.75% Cyfuthrin 0.15%	susceptible
An. dirus	9		“
An. aconitus	30		“
An. varuna	30		“
An. annularis	30		“
An .philippinensis	30		“
An. maculatus	30		“
An. kochi	30		“
An. vagus	30		“

Table 4 shows that All collected main vector *An. minimus* and *An. dirus*, Secondary vector *An. aconitus, An. varuna, An. annularis, An. philippinensis, An. maculatus, An. kochi. An. vagus* were found susceptible to WHO recommended insecticides impregnated paper.

Table 5. Bio-efficacy of impregnated mosquito nets in the study periods by cone test method

Used long lasting insecticidal nets	Ohnpinkone		Bwedarkone Control	
	Tested nets	Bio-efficacy (>80%)	Tested nets	Bio-efficacy (>80%)
PermaNet-2 (Thailand)	10	2(20%)	10	7(70%)
Yarkool (China)	10	2(20%)	10	5(50%)
Yahei (China)	10	2(20%)	10	7(70%)
Total	30	6(20%)	30	19(63.33%)

Table 5 shows that bio-efficacy (WHO recommended efficacy: Above 80% to 100% Mortality) of distributed three brands of used Long Lasting Insecticidal Nets (LLINs) in Ohnpinkone and Bwedarkone were found 6(20%) and 19(63.33%). Bwedarkone LLINs nets were found higher bio-efficacy than Ohnpinkone ward. Although the bio-efficacy was lower than the WHO recommended efficacy. All the distributed used and unused LLINs mosquito nets were found expiries their usable date.

4. Discussions

Malaria is a public health problem in Myanmar till now due to the morbidity is high in hard to reach areas. Malaria morbidity is found every month in this area. *Plasmodium vivax* cases were found high prevalence in some areas in Myanmar [7,31]. Therefore, there is a need to control malaria morbidity as well as man vector contact. *Anopheles minimus* is also a major vector of malaria in plain and foothill areas and contributing to 85-90% malaria infection after biting to nan immune person in Myanmar [10,21,32,33]. *An. annularis, An. culicifacies, An. maculatus, An. aconitus*, and *An. hyrcanus* are secondary vectors of malaria in Myanmar [6,10]. These mosquitoes were found sporozoites positive by ELISA method [21,32-34].

The monthly occurrence of *Anopheles* mosquitoes, potential vectors and susceptibility of status of mosquitoes were done in Ohnpinkone and Bwedarkone wards. In Ohnpinkone and Bwedarkone wards, a total of 3236 and 3019 *Anopheles* mosquitoes were collected within 9 months (December to October) of this n=2450 by cattle bait, 301 by outdoor and 486 by indoor light traps were caught in Ohnpinkone wards and 2639 by cattle bait, 154 by outdoor and 227 by indoor light traps were collected in Bwedarkone ward. A total of 22 *Anopheles* mosquito species were collected *An. barbirostris* was found to be highest number in both wards followed by *An. philippinensis* and lowest was observed *An. theobaldi* in both areas. *Anopheles minimus* was found to be n=214 in Ohnpinkone and 163 in Bwedarkone wards of this 72 in Ohnpinkone and 67 in Bwedarkone wards by Indoor light trap method and larvae were abundantly found in water well, water pools and sand pools in both areas. *An. minimus* is a main vector of malaria in Myanmar [10] and larvae were abundantly found in slowly running water, foot hill areas, rice field and water well in Oktwin Township, Tangoo Township in Bago Region, Ye Township in Mon State and Kamamaung Township in Kayin State [22,32,35,36]. Only 9 *An. dirus* in Ohnpinkone and 1 *An. dirus* in Bwedarkone were collected by Indoor light traps method and larvae were collected in water pools, sand pools and water wells in both

areas. *An. dirus* is a main vector of malaria and abundantly found in Bago Yoma mountain range and larvae were found rock pools, domestic water wells in Mon, Kayin State and Taninthayi Region [19,32,37,38]. One *An. minimus* was found to be *P. falcipaum* circumsporozoite antigen positive by ELISA method. Same sporozoite positive result has been found in Thabwewa village Bago Yoma mountain range, Katinehtit village Kayin State, Pyinoolwin Township Mandalay Region [22,32,37]. *An. dirus* was not sporozoite positive in both areas although other researchers observed that *An. dirus, An. vagus, An. minimus* were found sporozoite positive in Oktwin Township, Ghopinkauk Township Bago Region, *An. dirus* was sporozoite positive in Yephyu Township Taninthayi Region, Kamamaung Township Kayin State and *An. dirus, An. minimus, An. kochi, An. culicifacies, An. aconitus* and *An. maculatus* were sporozoite positive in Ann Township Rakhine State and Buke pin Township Tanintaryi Region , *Anopheles maculatus* was *Pf* sporozoite positive in Taikkyi Township, Yangon Region, in Myanmar [19,32-34,39]. *An. dirus*, *An. minimus An. philippinensis, An. maculatus* and *An. epiroticus* are main vectors of malaria in Thailand [25,40] although in malaria endemic region of Sukabumi, West Java, Indonesia, all collected *Anopheles* mosquitoes 7770 specimens were tested for circumsporozoite antigen by ELISA test was found negative for sporozoite [41].

Highest number of *Anopheles* mosquitoes were collected in post monsoon months in October (n=567) in Ohnpinkone and in September (n=727) it may be due to the fact that high number of water pockets were remaining in post monsoon in both areas and these are favourable for breeding of gravid *Anopheles* female. Same result has been found in Thabwewa village Bago Region and Katinehtit village Kayin State [22,32]. Although Stoops and his party observed that high density of *Anopheles* mosquitoes were collected in most in October in Sukabumi West Java, Indonesia [41]. Maung Maung Mya and his associates, revealed that *An. dirus* were collected in high density in June to July and Sept to October Kamamaung Township Kayin State and *An. minimus* was found monthly while the density was high in the month of July in Pyinoolwin Township Mandalay Region and Katinehtit village Kayin State [22,37]. Other researcher revealed that main vectors of *An. dirus* and *An. minimus* were collected abundantly in monsoon period Oktwin and Yephyu Townships [19,32]. In India main vector *An. culicifacies* and *An. fluviatilis* were abundantly collected in monsoon period [11,12]. The density of main vectors is varying in different malaria endemic regions, depend on the species of mosquitoes, climate, biology and ecology of these mosquitoes. In Laos, the three primary vector species such as *An. dirus s.s., An. maculatus s.s.* and, *An. minimus s.s.,* identified by Marcombe et al. [42].

In the present study the density of *Anopheles* mosquitoes by light traps catch were differ in different months, in outdoor light trap catch, highest number of *Anopheles* mosquitoes were collected in Ohnpinkone (109) than Bwedarkone (24) wards in April, and in January, there was not collected any *Anopheles* mosquitoes in light traps outdoor and indoor catch in Ohnpinkone wards and followed by July (53). In Bwedarkone the highest number of *Anopheles* mosquitoes were collected in March (38) followed by June and July (33) each respectively. and lowest was collected in August in both wards. Tun Lin et al. [32] observed that high number of *Anopheles* mosquitoes were collected by light trap outdoor in Thabwewa village although high number of *An. dirus* was collected in outdoor than indoor light traps catch and human bate outdoor collection method. Same result has been found in Kamamaung Township Kayin State and Yephyu Township Taninthayi Region [19,36].

In indoor catches, *Anopheles* mosquitoes were found gradually rising from February to August in Ohnpinkone ward but the highest number of *Anopheles* was collected in August (126) in Ohnpinkone ward, and in June (68) in Bwedarkone ward second most was found in June and July (107) each in Ohnpinkone and July (51) in Bwedarkone and lowest number was collected in February (4) and August (2) in Ohnpinkone and Bwedarkone respectively. *Anopheles minimus* was collected abundantly by light trap indoor and human bait indoor methods in Thabwewa village Bago Region, Yephyu Township Taninthayi Region, Pyinoolwin Township Mandalay Region, Katinehtit village in Kayin State and Ye Township Mon State [19,15,22,32,37,38].

When compared with indoor mosquitoes in both wards Ohnpinkone was found higher than Bwedarkone except January and February in indoor catch. Maung Maung Mya and associates revealed that main vector *An. dirus* and *An. minimus* were abundantly collected in monsoon periods in indoor catch in Kayin State and secondary vector *An. culicifacies* was abundantly collected in Poukkaung Township Bago Region by outdoor catch and *An. minimus* was abundantly collected in Pyinoolwin Township Mandalay Region in monsoon period in July by indoor light trap and cattle bait catch [15,37]. Other researchers observed that in India *Anopheles culicifacies* is in high densities (per man-hour densities) and is predominantly a zoophilic species, and indoor-resting collections from cattle sheds are generally more than those from human dwellings in monsoon period [15, 43-45].

Although *An. minimus* was collected every month in both Ohnpinkone and Bwedarkone wards. In Ohnpinkone ward *Anopheles minimus* was found suddenly increased from February and highest pick was found in April then the density of *An. minimus* was decreased to June. In Bwedarkone ward *An. minimus* was gradually increased from December to June then suddenly decreased to August and continuously decreased to October. *An. dirus* was found small peak on June in both areas although slightly high peak was found in Ohnpinkone ward. In Laos, *An. dirus* s.s., *An. maculatus* s.s. and, *An. minimus* s.s. represented 89, 36 and, 33% of the total mosquitoes of the *Dirus* and *Minimus* complexes and *Maculatus* group, respectively were collected in monsoon months [42]. In the *Maculatus* group, *An. rampae* represented 34% of the total but this zoophilic species is not a malaria vector [46]. In contrast, within the same group *An. sawadwongporni* represented 14% of the total and is considered as a very efficient vector in Thailand mostly found in monsoon months [47]. Within the *Minimus* complex, *An. aconitus* accounted for 57% of the total and represented more than 10% of the total number of the mosquitoes collected in same period. It should be observed that this species is morphologically closely resemble *An. minimus* but is not part of the *Minimus* complex, and can be listed as part of the *Funestus* Group. This species is highly zoophilic and exophagic in nature [48,49].

A total of 127 and 119 water sources were detected for *Anopheles* larvae in both Ohnpinkone and Bwedarkone wards of this 60.63% and 52.94% of the water sources were *Anopheles* larvae positive. Highest density of *Anopheles* larvae was collected in Rice field water sources followed by water pools and lowest was observed in creeks and wells in both Ohnpinkone and Bwedarkone wards. Main vector *An. dirus* larvae were collected in water wells only and higher number was collected from Ohnpinkone than from Bwedarkone wards. Other researchers mentioned that *An. dirus* larvae were abundantly collected in domestic water well in coastal areas of Mon and Taninthayi Region and rock pools in hilly areas of Thabwewa village Oktwin Township Bago Region and Katinehtit villages, Kamamaung Township Kayin State were agreed with the present study [10,19,32,37].

Main vector *An. minimus* larvae were collected in all water sources such as water wells, valleys, rice fields, water pools and sand pools water except in creeks in both areas. Highest number of *An. minimus* larvae were collected in wells followed by sand pools and lowest was collected in valleys in Ohnpinkone ward although in Bwedarkone ward, the highest number of *An. minimus* larvae were collected in sand pools followed by water wells and lowest was observed in rice fields. Same result has been found in slowly running water in forest foot hill areas and plain areas of sand pools and water pools in Pyinoolwin Township Mandalay region and Oktwin Township Bago Region, Yephyu Township in Taninthayi Region and in Yunsalin Creek in Kamamaung Township, Kayin State [15,19,22,32,36,37]. Maung Maung Mya and his party observed that high density of *An. minimus* larvae were collected in domestic water wells in Ye Township in Mon State [38]. Secondary vector *An, annularis* is a local vector of malaria in Rakhine State and *An. maculatus*, *An. culicifacies*, *An. kochi* and *An. aconitus* were abundantly collected from Rakhine State and Bukpin Township Taninthayi Region and they were sporozoite positive [33].

All collected main vector *An. minimus* and *An. dirus*, and secondary vector such as *An. aconitus, An. varuna*, *An. annularis, An. philippinensis, An. maculatus, An, kochi*. *An. vagus* were found susceptible to Deltamethrin 0.05%, Cyfuthrin 0.15%, Permethrin 0.75% of WHO recommended insecticides impregnated paper. Same susceptibility result of *An. dirus* and *An. minimus* has been found in Thabwewa village Bago Region, Ye Township Mon State, Kamamaung Township Kayin State, Pyinoolwin Township Mandalay Region and other parts of Myanmar [15,32,37,38,50]. The susceptibility status of *Anopheles* species in Laos, the main vectors such as *An. dirus*, *An. maculatus* and *An. minimus* showed absent of pyrethroid resistance [51]. Although *An. minimus* was found resistant to Deltamethrin 0.05% and DDT4% in Thailand [40]. In India for indoor residual spraying, DDT, malathion and pyrethroids have been introduced in the malaria control programme in a sequential order. In 1959 *An. culicifacies* was reported resistant to DDT [52], to hexachlorocyclohexane [53] and in 1973 to malathion [54]. The differential development of resistance among the sympatric sibling species under similar selection pressure was observed [11,55-58]. In a recent review of the susceptible status of vector species to different insecticides, it is mentioned that *An. culicifacies* in the rural plains have exhibited widespread resistance [59] In 70% of the districts examined, this species has shown resistance to at least one insecticide especially in Chhattisgarh, Madhya Pradesh and Odisha [2].

The pyrethroids used to treat the ITNs have an exito-repellent effect, thus adding a chemical barrier to the physical one and enhancing personal protection by nets [60,61]. In the present study there are 4 kinds of mosquito nets were found in Ohnpinkone and Bwedarkone wards such as LLINs nets, cotton nets, Polyester nets and traditional nets, of this highest number of used LLINs nets were found low bio-efficacy (20%) and 63.33% because all the distributed LLINs nets in Ohnpinkone ward were used for 4 years and the expiry date was over 6 months and all the nets were washed over 15 washes. In Bwedarkone ward all the received LLINs nets were 3 years old and 10-15 washed and the nets were near to the expiry date. Therefore, the efficacies were differed between both wards. Same result has been found in Thanbyuzayat Township Mon State, the bio-efficacy of DAWA TANA LLINs nets were found 83.33% bio-efficacy and in Pyinoolwin Township Mandalay Region, the Yahei LLINs nets were found 90% bio-efficacy. In different malaria endemic areas in the country, other brands of 0-3 times washed LLINs nets such as ParmaNet 2.0, Yorkool, BASF, Net protect were found 100% sensitivity before the expiry date [62,63]. In

Western Kenya WHO resistance assays revealed that f1 offspring of *An. gambiae* samples were collected in nets in Bungoma were 94% and 65% resistant to deltamethrin and permethrin, respectively. Nets from Bungoma retained strong activity against a susceptible laboratory strain, but not against f1 offspring of field collected *An. gambiae* s. s. All *An. gambiae* s. s. samples collected in nets were homozygous for the Kdr genotype L1014S [64]. Ochomo and associates suggested that in areas with pyrethroid resistant vectors, LLINs with modest hole areas permit mosquito entry and feeding, providing little protection against the vectors. LLIN formulations develop large holes within three years of use, diminishing their presupposed lifetime effectiveness [42].

5. Conclusions

Ohnpinkone and Bwedarkone words from Nanyin village were chosen for measuring bionomics potential vectors, susceptibility status of Anopheles mosquitoes. *Anopheles* mosquitoes were collected by cattle bait, indoor and outdoor CDC light traps methods. A total of 3236 and 3019 *Anopheles* mosquitoes consist of 12 species were collected from both Ohnpinkone and Bwedarkone wards. *An. barbirostris* and *An. philippinensis* were abundantly collected in both areas. Higher number of *Anopheles* mosquitoes were collected by Cattle bait followed by Light traps indoor method. Main vectors of *An. minimus* and *An. dirus* were collected in higher number in Ohnpinkone than Bwedarkone. High number of *An. minimus* was collected by cattle bait catching method and one *An. minimus* from Ohnpinkone was found sporozoite positive (0.47%). *An. dirus* was collected high number by indoor light traps in both wards. High number of *Anopheles* mosquitoes were collected in October in Ohnpinkone and September in Bwedarkone. Indoor light traps catch was found high peak in August and June as well as by outdoor was found April and March in both wards respectively. *An. minimus* was found highest in April in Ohnpinkone and June in Bwedarkone. *An dirus* was found higher in June in both areas. Primary and secondary vectors were found susceptible to WHO recommended insecticides. All tested LLINs nets were found lower efficacy and main vector *An. minimus* was collected year round in high density as well as sporozoite positive and *An. dirus* were available in both areas. Therefore. study suggest that, proper treatment and control of *Anopheles* mosquito vectors using Residual Insecticide Spray (RIS) and changing new LLINs nets with old nets should be done. Improving awareness, malaria knowledge and practices should be given by expert. If cannot supply new LLINs nets, should be treated to old LLINs with deltamethrin according to WHO guideline to prevent malaria transmission in both areas.

6. Acknowledgements

This study was supported by ICEMR Asia. I am thankful to our Director (ICEMR) who allowed me to do the research. And also, I am also highly thanks to authority concerns who were permitted and helpful to do research works in their wards. I am thankful to the staffs of ICEMR who were helpful to the research works till completion.

Declarations

Source of Funding

This research work did not receive any grant from funding agencies in the public or not-for-profit sectors.

Competing Interests Statement

The authors declare no potential conflicts of interest concerning the research, authorship, and publication of this article.

Consent for publication

The authors declare that they consented to the publication of this research work.

Authors' Contributions

All authors equally contributed to data collection, survey, analysis and paper drafting.

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